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Isolation and determination of amines and quaternary ammonium ions as complexes*

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In this survey the following papers will be discussed and will be referred to by Roman numerals.

I. G. Schill and B. Danielsson. Photometric determination of quaternary ammonium compounds with hexanitrodiphenylamine. I. *Anal. Chim. Acta* **21**, 248 (1959)

II. G. Schill. Photometric determination of quaternary ammonium compounds with hexanitrodiphenylamine. II. *Anal. Chim. Acta* **21**, 341 (1959)

III. G. Schill and M. Marsh. Photometric determination of amines and quaternary ammonium compounds with bromothymol blue. I. *Svensk Farm. Tidskr.* **67**, 385 (1963)

IV. G. Schill. Photometric determination of amines and quaternary ammonium compounds with bromothymol blue. Part 2. Association of bromothymol blue in aqueous solutions. *Acta Pharm. Suecica* **1**, 101 (1964)

* Inaugural dissertation.

V. G. Schill. Photometric determination of amines and quaternary ammonium compounds with bromothymol blue. Part 3. Partition of bromothymol blue between water and methylene chloride. *Acta Pharm. Suecica* 1, 169 (1964)

VI. G. Schill. Photometric determination of amines and quaternary ammonium compounds with bromothymol blue. Part 4. Extraction constants and calculation of extraction conditions. *Acta Pharm. Suecica* 2, 13 (1965)

VII. G. Schill. Photometric determination of amines and quaternary ammonium compounds with bromothymol blue. Part 5. Determination of dissociation constants of amines. *Acta Pharm. Suecica* 2, 99 (1965)

VIII. G. Schill. Photometric determination of amines and quaternary ammonium compounds with bromothymol blue. Part 6. Association in aqueous solution between bromothymol blue and quaternary ammonium ions. *Acta Pharm. Suecica* 2, 109 (1965)

IX. G. Schill, R. Modin and B.-A. Persson. Extraction of amines as complexes with inorganic ions. *Acta Pharm. Suecica* 2, 119 (1965)

The determination of aliphatic amines and quaternary ammonium ions is one of the most important tasks in pharmaceutical analysis. This is because most of the substances with strong physiological effects used in pharmaceutical products contain these functional groups. The isolation and determination methods used must be suitable for low concentrations, since the substances are often present in extremely small amounts.

In many cases, isolation is achieved by chromatography, or by other methods utilizing differences in migration velocity. The amines often migrate with the organic solvent as bases, whereas quaternary ammonium ions with high equivalent weight or containing long-chain groups can give migrating compounds with suitable anions. This can also apply to cations of amines with corresponding properties. However, in many methods the nature of the migrating substance is unknown.

The compounds, soluble in organic solvents, which are formed between the ammonium ions and organic or inorganic ions, have been denoted as ion association complexes or ion pairs. In the papers reviewed here, both these terms are used, without a closer study of possible differences in the binding between the components.

The association complexes have also been utilized in the determination methods. Several organic protolytes give anions with high absorptivity, which form complexes with the ammonium ions, extractable from aqueous solutions with organic solvents. If suitable anions are used, complexes with a molar absorptivity considerably higher than 10^4 can be obtained, and sometimes a further increase in absorbance can be achieved by reactions in the organic phase. Due to the high absorptivity,

extremely small concentrations of amines or quaternary ammonium ions can be determined.

The complex-forming reagents used belong mainly to three groups, *i. e.*, nitro compounds such as dipicrylamine, sulphonphthaleins like bromothymol blue, and azo compounds such as Tropaeolin 00. The usefulness of the reagents is governed by the acid dissociation constants and partition coefficients of the reacting components, as well as by the stability and partition coefficient of the complexes formed. By varying the reagent, organic solvent and pH, it is possible to obtain extraction conditions which make the method to some extent specific for a group of substances.

The stoichiometry of the complex must be well defined, a fact which must be taken into account especially when the reacting components can ionize with several valences.

Interfering effects of foreign substances is another important problem inherent in the methods. Extraction can be disturbed by substances which bind the reacting components in the aqueous phase, or which form extractable compounds.

Symbols

In this review the following symbols, not defined in the text, are used:

$[\ ]_{org}$, $[\ ]$ = concentration in the organic and aqueous phase, respectively

C_{org}, C = total concentration in the organic and aqueous phase, respectively

$$k_{d(M)} = \frac{[M]_{org}}{[M]} = \text{partition coefficient}$$

$$K'_{HM^+} = \frac{a_{H^+} \cdot [M]}{[HM^+]} = \text{apparent acid dissociation constant}$$

Determination of quaternary ammonium ions with hexanitrodiphenylamine

Hexanitrodiphenylamine has been used in the gravimetric determination of quaternary ammonium compounds (1). It was found that complexes can be extracted with chloroform and methylene chloride, as illustrated by the formula

$$A^+_{aq} + He^-_{aq} = AHe_{org}$$

in which A^+ denotes a monovalent quaternary ammonium ion, and He^- the anion of hexanitrodiphenylamine, HHe. Conditions associated with negligible extraction of HHe were calculated from its acid dissociation constant and partition coefficients (I: Fig. 6).

In strongly alkaline medium, He^- reacts with base, giving a product presumed to be a carbinol

$$He^- + OH^- = HeOH^{-2}$$

The reaction can be followed by the changes in absorptivity. It is considerably slower than ordinary protolysis and seems to be reversible, although irreversible reaction products are also formed, the amounts of which increase with time and alkali concentration (II: Fig. 1, Table I).

The extraction of some complexes is complicated by their limited solubility in methylene chloride and low solubility in water. In most cases, these difficulties can be avoided by using a strongly alkaline aqueous phase, where no precipitation occurs, due to low $[He^-]$. Since $HeOH^{-2}$ is formed fairly slowly, extraction during a short time immediately after addition of HHe to the extraction mixture will give a higher concentration of complex in the organic phase than extraction after the equilibrium concentration of $[He^-]$ is attained. Using this technique, a few extractions with a limited excess of HHe suffice for complete removal of A^+ from the aqueous phase.

The complexes show varying stability in the organic phase, reflected in inconstant molar absorptivity and even precipitation. Addition of an excess of tetrabutylammonium iodide will, in all cases, produce good stability and an absorptivity of $10^{4.50}$ per equivalent of He^- (II: Table VII).

The method was tested with 20 quaternary ammonium ions, most of them used in pharmaceutical products, and containing 1—3 quaternary groups (II: Tables III and IV). Extraction from a strongly alkaline aqueous phase gave satisfactory results in most cases. Phenols and phenol esters required pH 11 of the aqueous phase, as well as some ions containing hydrophilic groups, such as choline and choline esters.

The blanks found were negligible, but at pH 11 increasing values were recorded with increasing concentrations of Na^+ and K^+. No interference by amines was observed in a few cases studied (II: Table IX). The insolubility of the complexes of the bisquaternary ammonium ions in chloroform was utilized in a method by which monoquaternary and bisquaternary ammonium ions in mixture could be determined selectively (II: Table X).

Determination of amines and quaternary ammonium ions with bromothymol blue

Bromothymol blue (BTB) has been used (2) in the determination of amines, as well as of quaternary ammonium ions, as demonstrated by the reaction

$$HA^+_{aq} + HB^-_{aq} = HAHB_{org}$$

in which HA^+ denotes an ammonium ion and HB^- the monovalent anion

of BTB. The extracted complex has the molar absorptivity, $\varepsilon^{410} \approx 10^{4.3}$, but somewhat inconstant results were obtained. Addition of an excess of tetrabutylammonium hydroxide (TBAH) produces transformation to a complex containing BTB as a divalent anion, B^{-2}, with a molar absorptivity, $\varepsilon^{630} = 10^{4.69}$. A suitable solvent for this reaction is water-saturated methylene chloride, the water content being essential for stability of the absorptivity (III: Table 2).

The extraction procedure is frequently complicated by the formation of emulsion, which increases the time necessary to attain equilibrium. It has also been observed that BTB and the complex can be adsorbed to the interface which, on incomplete phase separation with emulsion residues, causes large variations in the absorbance of the organic phase (III: Table 6). To avoid these errors, phase separation by centrifugation is used in the extraction procedure. Precipitation of the complex in the aqueous phase on addition of BTB has been observed and can, due to slow attainment of equilibrium, give rise to errors. These can be prevented if extraction is started before excess of BTB is added.

Calculations using the second acid dissociation constant and a conditional partition coefficient of BTB showed that the complexes had to be extracted at pH $\geq$ 7.5, if low blanks due to uncomplexed BTB were to be obtained. In this pH range and with a BTB excess of > 100 %, a large number of amines and quaternary ammonium ions could be almost completely extracted in one operation (III: Tables 7 and 8). A test of interfering substances showed that increasing concentrations of K^+ and NH_4^+, and to some extent of Na^+ as well, gave increasing blanks (III: Table 5).

When testing the proposed method, observations were made that could not be explained by available data on BTB and the complexes, primarily the incomplete extraction of some substances (III: Tables 9 and 10) and the disturbances by inorganic cations. Since the method seemed to afford good possibilities of determining extremely small amounts of many amines and quaternary ammonium ions, and could be considered representative of several similar procedures, a more thorough study was made of the systems used.

Bromothymol blue in aqueous solution

The first acid dissociation constant of BTB was determined by solubility studies at the ionic strengths 1.0 and 0.10, using variations in $[H^+]$ and $[Na^+]$. It is reported as a stoichiometric constant (IV: Table 2), since part of the determinations were performed in solutions with an acidity which made determinations of a_{H^+} uncertain. The second dissociation constant was obtained by photometric measurements. It is given as an apparent constant in solutions with C_{Na^+} ranging from 0.015 to 1.05 (IV: eq. 7, V: Table 3).

Increasing concentrations of BTB were found to give rise to association, which is in accordance with observations made with bromophenol blue (3) and other phenol sulphonates (4, 5). The association products formed depend on the concentration of the monovalent anion, HB^-, and of Na^+. No evidence was found of association of the divalent anion, B^{-2}.

At $C_{Na^+} \leq 0.010$, association products without colloidal properties are formed. The formation of dimers and tetramers of HB^- is likely, as seen from a graphical computation (IV: Figs. 6 and 7). Approximate values of the dimerization and tetramerization constants were determined. The relation between total concentration of HB^- and the concentrations of monomer, dimer and tetramer is given in IV: Fig. 8. The assumptions made regarding association were supported by the results of absorbance measurements and solubility determinations.

At C_{Na^+} between 0.010 and 0.07, association products of colloidal character are formed, as obvious from increased viscosity and turbidity. This is followed by an absorptivity increase at 284 mμ, varying with C_{Na^+} and strongly temperature-dependent (IV: Figs. 9 and 10). Using values of the association constants and the absorptivities of ionic components obtained at $C_{Na^+} = 0.010$, the equivalent absorptivity at 284 mμ of NaHB in the micelles was calculated (IV: Tables 7—9). The values of the equivalent absorptivity were not altogether constant, which may have been due to variation in the constitution of the micelles with C_{Na^+}.

The critical micelle concentration (CMC) at different C_{Na^+} was estimated from the relation between C_{HB^-} and the micelle concentration, calculated with the use of the association constants (IV: Fig. 13). At higher C_{Na^+}, where dimerization and tetramerization were negligible, CMC could be determined by the relation between C_{HB^-} and the absorptivity at 284 mμ (IV: Fig. 12). The relation between log CMC (expressed in $[HB^-]$) and log C_{Na^+}, which is linear, permits an approximate calculation of the maximal $[HB^-]$ at different C_{Na^+} (IV: Fig. 14).

When C_{Na^+} is > 0.07, precipitation of NaHB can occur, and the maximum HB^- will be calculable from the solubility product (IV: Table 2).

The found relations between C_{Na^+} and $[HB^-]$ have important consequence in the determination of amines and quaternary ammonium ions. The determination must always be made with the same pH in test sample and blank. This requires a fairly high capacity of the buffer system used, which will probably have a cation concentration of > 0.01 M. In this concentration range, increasing cation concentration will give decreasing $[HB^-]$, and this can reduce the complex extraction to such an extent that the determination will be impossible. The choice of buffer system must therefore be a compromise between the desires of high capacity and low cation concentration. It must, however, be emphasized that Na^+ is the only cation thoroughly studied, but preliminary experiments with other ions gave larger disturbances.

Partition of bromothymol blue in the presence of Na^+

The partition ratio of BTB was found to vary strongly with C_{Na^+} of the aqueous phase, and the absorbance of the organic phase indicated that BTB was to some extent, varying with C_{Na^+}, present in the organic phase in ionized form. Absorbance measurements and flame-photometric determinations of sodium in the organic phase suggested that this phase contained BTB as acid, H_2B, and as NaHB in monomeric and polymeric forms. Analogous observations have been reported with other sodium sulphonates (6, 7, 8).

The absorptivity of the components in the organic phase was determined, and it was found that the total concentration of NaHB can be estimated by absorbance measurements (V: Table 1). Partition experiments with C_{Na^+} ranging from 0.05 to 1.05 verified the assumption of polymerization of NaHB in the organic phase. NaHB in the monomeric form could be extracted at $C_{Na^+} \leq 0.156$, and an approximate value of the extraction constant

$$E_{NaHB} = \frac{[NaHB]_{org}}{[Na^+] \cdot [HB^-]}$$

was obtained by graphical computation (V: Fig. 1).

Partition at C_{Na^+} ranging from 0.31 to 1.05 gave results indicating that the polymeric NaHB in the organic phase contains more than 10 monomers (V: Fig. 2). Water seems to be essential for the stability of the polymers, and determinations indicated a content of about 30 molecules of water per molecule of NaHB (V: Table 7).

The partition coefficient of H_2B is independent of the concentration of H_2B and HB^- in the phases (V: Fig. 3). Since K'_{H_2B} is not known at the ionic strengths used in the experiments, $k_{a(H_2B)}/K'_{H_2B}$ was computed.

The values of E_{NaHB} and $k_{a(H_2B)}/K'_{H_2B}$ can be used in the calculation of the blank in the determination method given in paper III by the formula

$$C_{blank} = \frac{C_{BTB}}{1 + 10^{pH-7.18}} (C_{Na^+} \cdot 0.2 + 10^{5.35-pH})$$

The expression is valid in such ranges of C_{Na^+} and $[HB^-]$ that no association of HB^- occurs in the aqueous phase and no polymers of NaHB are formed in the organic phase, *i. e.*, $C_{HB^-} \leq 2 \cdot 10^{-4}$ and $C_{Na^+} \leq 0.15$. If the aqueous phase contains Na^+ and HB^- in concentrations above these ranges, increased blanks are obtained, and at high C_{Na^+} determinations are impossible, due to the high partition ratio of NaHB.

Extraction of bromothymol blue complexes

BTB was found to give extractable complexes in the form of monovalent as well as divalent anion, which is in analogy with observations made with bromophenol blue (9) and Tropaeolin 00 (10). The properties of the complexes on extraction are given by the extraction constant, E, which with the HB^- complex of an ammonium ion, HA^+, is defined as

$$E_{HAHB} = \frac{[HAHB]_{org}}{[HA^+] \cdot [HB^-]}$$

The extraction constants were calculated from total concentrations of the reacting components, making corrections for side-reactions by α-coefficients according to Ringbom (11)

$$E_{HAHB} = \frac{C'_{BTBorg}}{C_{BTB} \cdot C_A} \cdot \frac{\alpha_{HA} \cdot \alpha_{HB}}{\alpha_{HAHB}}$$

C'_{BTBorg} is the total concentration of BTB in the organic phase, corrected for the extraction of H_2B and NaHB. The side-reactions which have to be taken into account with a monoamine are protolysis of BTB and amine in the aqueous phase, and extraction of the amine as base (VI: eq. 16 and 18).

When the amount of amine present in the aqueous phase is negligible compared with the amount extracted as base, another constant defined as

$$k'_e = E_{HAHB}/k_{d(A)} \cdot K'_{HA^+}$$

can be used instead of E_{HAHB}.

With diamines, two HB^- complexes, HAHB and $H_2A(HB)_2$, can be extracted. The extraction constants were obtained by graphical computation (VI: Fig. 1). In cases where the uncomplexed amine is mainly in the organic phase, k'_e and k''_e are used instead of E_{HAHB} and $E_{H_2A(HB)_2}$. k''_e is defined as

$$k''_e = E_{H_2A(HB)_2}/k_{d(A)} \cdot K'_{H_2A^{+2}} \cdot K'_{HA^+}$$

Extraction of complexes containing the divalent anion of BTB was observed only with some monoquaternary ammonium ions, and extraction of complexes containing HB^- occurred simultaneously (VI: Table 5).

The extraction constants of 30 mono- and diamines and 21 mono- and bisquaternary ammonium ions were determined (VI: Tables 2—6). The

substances chosen for this study were intended to represent properties common in substances used in pharmaceutical products. With most amines, E as well k_e values are given, the latter being calculated with the use of the partition coefficients and acid dissociation constants (VI: Tables 1 a and 1 b). It must, however, be emphasized that exact knowledge of these data is not necessary in many determinations of partition properties of the complexes. In cases where k'_e values can be used, *i. e.*, when the amount of uncomplexed amine in the aqueous phase is negligible, k'_e can be determined directly (VI: eq. 87 and 88). If the amine is completely protolyzed in the aqueous phase and no extraction of amine as base occurs, the calculation of E is made with correction only for the protolysis of BTB.

The extraction constants are used in the estimation of the degree of extraction as complex, P. The complete expressions of P are fairly complicated (VI: eq. 49, 53), since they give, not only the influence of C_{BTB}, pH and phase volumes, but also corrections for side-reactions of different kinds. It is, however, possible to cover the most important classes of substances by a few simpler equations (VI: Table 8).

Suitable extraction conditions for different kinds of substances can be estimated from the P equations. In general, extraction at pH 7.5 with as high $[HB^-]$ as possible will afford the most complete extraction. Diamines, extracted as HAHB, are exceptions and the optimal pH is obtained by graphical computation (VI: Fig. 2). Using the extraction constants, conditions for selective extraction of one component in a mixture of several complex-forming cations can also be calculated (VI: eq. 81 and 82).

No simple relation was found between extraction constant and constitution of the heterogeneous group of amines and quaternary ammonium ions used in the present study. In a few cases, homologous compounds were studied, and an almost linear increase in log E with the number of carbon atoms in the cation was found (VI: Fig. 4 and 5). As a rule, 7 or more carbon atoms per nitrogen atom are needed to give such a high extraction constant that a quantitative determination of the cation is possible under the conditions normally used. The presence of hydrophilic groups or too high acid dissociation constant of an amine can, however, decrease the degree of extraction to such an extent that the determination will be impossible.

Amines with aromatic character gave, in general, higher extraction constants than aliphatic or non-aromatic cyclic compounds (VI: Fig. 6 and 7). A distinct decrease in E was given by phenolic groups, and to a smaller extent also by alcoholic hydroxyls. Several cyclic ethers had lower extraction constants than expected from their number of carbon atoms. Most monoamines gave fairly constant values of the relation $E_{HAHB}/k_{d(A)}$ (VI: Fig. 3) indicating that differences in extraction constants were due mainly to different hydrophobic properties of the amine.

Partition coefficients and dissociation constants of amines

The estimation of such side-reactions of the amines as protolysis in the aqueous phase and extraction of the amine as base required determination of partition coefficients and dissociation constants. In most cases, the results are presented as $k_{d(A)} \cdot K'_{HA^+}$ or $k_{d(A)} \cdot K'_{H_2A^{+2}} \cdot K'_{HA^+}$, which provides the information necessary for the side-reaction correction (VI: Tables 1 a and 1 b).

The calculation of partition coefficients required knowledge of the dissociation constants of the amines, and in most cases values in the literature were available. Determinations had to be made in a few cases, *i. e.*, chloroquine, chlorpromazine, 1,10-diaminodecane, ABOB and promazine (VII). The methods used were variation in solubility, partition ratio and absorbance in UV with pH.

Bromothymol blue complexes in aqueous solution

In determination of the extraction constants, the complex concentration in the aqueous phase was assumed to be negligible. With increasing concentrations of quaternary ammonium ions, decreasing extraction constants were observed, due to association between the quaternary ammonium ion and the anions of the BTB system in the aqueous phase. Similar association has been observed between amines and Tropaeolin 00 (12), and between quaternary ammonium ions and methyl orange (13).

The experiments were performed with tetramethyl-, tetraethyl- and tetrapropylammonium ions. The association was assumed to comprise the complexes AHB, AB^- and A_2B, the presence of AHB and A_2B being known from the extraction of complexes. The formation of AB^- was studied by partition experiments, and the association constant was obtained by graphical computation (VIII: Figs. 1—3).

The associations were also investigated with the help of the conditional acid dissociation constant of HB^-, defined as

$$\frac{a_{H^+} \cdot C_B}{C_{HB}} = K^x_{HB^-}$$

The total concentration of B^{-2} in the solution, C_B, was determined by absorbance measurements, and the association constant of AHB could be calculated from the variation in $K^x_{HB^-}$ with $[A^+]$ (VIII: Fig. 5). Approximate values of the association constant of A_2B were obtained from the same experiments.

Partition coefficients of the complexes were calculated from the association and extraction constants (VIII: Table 1). They show that the increasing extraction constants obtained with increasing number of carbon atoms are due mainly to the rise in $k_{d(AHB)}$, *i. e.*, the complex becomes more hydrophobic.

Extraction of amines as complexes with inorganic anions

Many amines and quaternary ammonium ions give association complexes with inorganic anions, soluble in chloroform or methylene chloride (14, 15, 16, 17, 18). A study of the partition properties of the complexes was made with the object of obtaining a basis for discussion of their use in partition chromatography, and of their influence in other extraction processes.

The amines were chosen so that ions pairs with highly different partition properties were obtained. They are defined by a conditional extraction constant (X denotes the inorganic anion)

$$E^{x}_{HAX} = \frac{C_{HAXorg}}{C_A \cdot C_X}$$

The determinations of the conditional extraction constants were made with chloroform as the organic phase, in such pH ranges that extraction of the amines as bases was avoided. The change in log E^{x}_{HAX} with the anion was of the same magnitude in all the amines tested. Thus, ion pairs containing I^- or SCN^- gave considerably higher extraction constants than ClO_4^-, Br^- and NO_3^-, whilst the lowest values were obtained with Cl^- (IX: Fig. 1). It is possible that this regularity will simplify the choice of anion for a wanted extraction or separation.

Dimerization in the organic phase was observed with lidocaine iodide and thiocyanate, and the dimerization constants were determined graphically (IX: Fig. 2). Codeine gave a decreasing extraction constant with increasing anion concentration, which may indicate association in the aqueous phase.

The ion pairs represent an effective way of extracting amines from aqueous solution, if the extraction constant is $\geq 10^{2.0}$. The disturbances in the bromothymol blue method, which may be caused by these complexes, can be calculated with the help of α-coefficients (IX: eq. 14 and 16). Under normal extraction conditions, none of the complexes discussed gave rise to errors.

The conditions under which these complexes can be used in partition chromatography are discussed, and the following criteria are stressed. Sample concentrations causing polymerization in the organic phase must be avoided, and the acid concentration of the aqueous phase must be so high that no partition of the amine as base occurs. The concentration of the complex-forming anion in the aqueous phase must be adapted to the sample concentration, so that variations in anion concentration during the chromatographic procedure are negligible (IX: eq. 20).

Partition chromatography experiments, performed with systems calculated according to the principles mentioned, resulted in chromatograms with symmetrical peaks, and retention volumes in fairly good agreement with the estimated ones (IX: Fig. 3).

Chromatography of amines as association complexes provides a wide variation of separation possibilities under easily calculable conditions, and seems to be superior to methods by which the amine migrates as a base.

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